

1     1.     A method for determining the concentration of chloride ions in samples,  
2 comprising:

3             preparing an enzyme reagent, said enzyme reagent including:

4                      $\alpha$ -amylase that is substantially calcium-free; and

5                     an  $\alpha$ -amylase activity detecting substrate; and

6             combining the enzyme reagent with sodium ion and a sample containing  
7 chloride ion to be assayed, the sodium ion being present in a higher concentration  
8 than said chloride ion;

9             assaying the quantity of  $\alpha$ -amylase <sup>activity</sup> formed due to the presence of sodium ions  
10 and chloride ions in said sample; and

11             determining the quantity of said chloride ions by reference to said assay of  $\alpha$ -  
12 amylase <sup>activity</sup>

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14     2.     The method according to claim 1, wherein calcium is removed from the  $\alpha$ -  
15 amylase that is substantially calcium-free by use of a chelating compound.

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17     3.     The method according to claim 1, wherein calcium is removed from the  $\alpha$ -  
18 amylase that is substantially calcium-free by use of a compound that forms a covalent bond  
19 with calcium.

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1           4.     The method according to claim 2, wherein said chelating compound is a  
2 member selected from the group consisting of ethylenediaminetetraacetic acid, trans-1,2-  
3 cyclohexanediamine-N,N,N',N'-tetraacetic acid, glycol ether diamine tetraacetic acid,  
4 iminotetraacetic acid, and diaminopropanetetraacetic acid.

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6           5.     The method of claim 2, wherein said chelating compound is  
7 ethylenediaminetetraacetic acid.

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9           6.     The method according to claim 1, wherein said  $\alpha$ -amylase activity detecting  
10 substrate is a member selected from the group consisting of 2-chloro-4-nitrophenyl- $\alpha$ -D-  
11 maltotrioxide, 4-nitrophenyl- $\alpha$ -D-maltopentaoside and  $\alpha$ -glucosidase, 2-chloro-4-  
12 nitrophenyl- $\beta$ -D-maltopentaoside and  $\alpha$ -glucosidase and  $\beta$ -glucosidase, 4-nitrophenyl- $\alpha$ -D-  
13 maltoheptaoside,  $\alpha$ -glucosidase, and 2-chloro-4-nitrophenyl- $\beta$ -D-maltoheptaoside and  $\alpha$ -  
14 glucosidase and  $\beta$ -glucosidase.

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16           7.     The method according to claim 6, wherein said  $\alpha$ -amylase activity detecting  
17 substrate is 2-chloro-4-nitrophenyl- $\alpha$ -D-maltotrioxide.

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19           8.     The method according to claim 1, wherein said sample is a bodily fluid  
20 sample.

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22           9.     The method according to claim 8, wherein said bodily fluid sample is  
23 selected from the group consisting of serum, plasma, or urine.

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1000 EAGLE GATE TOWER  
60 EAST SOUTH TEMPLE  
SALT LAKE CITY, UTAH 84111

1 10. The method of claim 1, wherein said sodium ion compound is sodium citrate.

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3 11. The method of claim 1, wherein said sodium ion compound is sodium  
4 acetate.

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1 12. A composition for use in determining the concentration of chloride ions in a  
2 fluid sample, comprising:  $\alpha$ -amylase that is substantially calcium-free, sodium ion, and an  $\alpha$ -  
3 amylase activity detecting substrate.  
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5 13. A composition as in claim 12 further comprising a compound capable of  
6 forming a chelate with a calcium ion and a calcium chelate compound.  
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8 14. A composition according to claim 13, wherein said compound capable of  
9 forming a chelate with a calcium ion is a member selected from the group consisting of  
10 ethylenediaminetetraacetic acid, trans-1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid,  
11 glycol ether diamine tetraacetic acid, iminotetraacetic acid, and diaminopropanetetraacetic  
12 acid.  
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14 15. A composition according to claim 13, wherein said compound capable of  
15 forming a chelate with a calcium ion is ethylenediaminetetraacetic acid.  
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17 16. The composition according to claim 13, wherein said calcium chelate  
18 compound is calcium-ethylenediaminetetraacetic acid.  
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1           17.    The composition according to claim 12, wherein said  $\alpha$ -amylase activity  
2 detecting substrate is a member selected from the group consisting of 2-chloro-4-  
3 nitrophenyl- $\alpha$ -D-maltotrioside, 4-nitrophenyl- $\alpha$ -D-maltopentaoside and  $\alpha$ -glucosidase, 2-  
4 chloro-4-nitrophenyl- $\beta$ -D-maltopentaoside and  $\alpha$ -glucosidase and  $\beta$ -glucosidase, 4-  
5 nitrophenyl- $\alpha$ -D-maltoheptaoside,  $\alpha$ -glucosidase, and 2-chloro-4-nitrophenyl- $\beta$ -D-  
6 maltoheptaoside and  $\alpha$ -glucosidase and  $\beta$ -glucosidase.

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8           18.    The composition according to claim 12, wherein said  $\alpha$ -amylase activity  
9 detecting substrate is 2-chloro-4-nitrophenyl- $\alpha$ -D-maltotrioside.

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11           19.    The composition of claim 12, wherein said sodium ion compound is sodium  
12 citrate.

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14           20.    The composition of claim 12, wherein said sodium ion compound is sodium  
15 acetate.

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1 (21.) A method of activating calcium-free  $\alpha$ -amylase for enzymatic activity  
2 comprising mixing chloride ion with calcium-free  $\alpha$ -amylase in the presence of excess  
3 sodium ion.

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1     22. A method for determining the concentration of sodium ions in samples,  
2 comprising:

3             preparing an enzyme reagent, said enzyme reagent including:

4                      $\alpha$ -amylase that is substantially calcium-free; and

5                     an  $\alpha$ -amylase activity detecting substrate; and

6             combining the enzyme reagent with excess chloride ion, and a sample  
7 containing sodium ion to be assayed, the chloride ion being present in a higher  
8 concentration than said sodium ion;

9             assaying the quantity of  $\alpha$ -amylase formed due to the presence of sodium ions  
10 and chloride ions in said sample; and

11             determining the quantity of said sodium ions by reference to said assay of  $\alpha$ -  
12 amylase.

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14     23. The method of claim 22, wherein a calcium-binding compound is combined  
15 with the enzyme reagent, the excess chloride ion, and the sample containing sodium ion to  
16 be assayed before the  $\alpha$ -amylase quantity is determined.

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18     24. The method of claim 22, wherein said calcium-binding compound is  
19 ethylenediaminetetraacetic acid.

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